REMARKS/ARGUMENTS

Claims 1-12 were pending. Claims 1-6 and 12 have been canceled as drawn to a non-elected invention. Claim 7 is amended, and new claims 13-16 are added. No new matter is added. Reconsideration of the rejections is requested.

Support for the amending language of Claim 1 may be found in the specification at paragraph 51 (recovering said Wnt protein from a lysate of said cell or from culture medium in which said cell is grown expressing a Wnt protein in a cell); at paragraph 7 (expressing a Wnt protein in a cell); and at paragraph 6 (wherein biological activity of said Wnt protein is maintained).

Support for the amending language of Claim 13 may be found in the specification at paragraph 13. Support for the amending language of Claim 14 may be found in the specification at paragraph 25. Support for the amending language of Claim 15 may be found in the specification at paragraph 26.

Applicants confirm the election of Group III, methods of isolating Wnt proteins. A dependent claim has been added, which is directed to the composition isolated by the method of Claim 7. Applicants respectfully submit that such a claim is properly included with Group III.

Claims 7-11 have been rejected under 35 U.S.C. 112, second paragraph. Applicants have amended independent Claim 7 to clarify the intended subject matter. In view of the above amendments and remarks, withdrawal of the rejection is requested.

Claim 7 has been rejected under 35 U.S.C. 102(e) as anticipated by Rodan *et al.*, U.S. Patent no. 5,780,291. Applicants respectfully submit that the cited art fails to teach a method of isolating a substantially homogeneous composition of Wnt protein where the biological activity of the protein is maintained.

Wnts are secreted factors that play important roles in embryonic development and in controlling the proliferation of stem cells. Prior to Applicants' invention, purification of active Wnt was a long sought goal, to provide protein for such uses. It was predicted that the Wnt proteins could act as growth factors that could be added to cells and direct them into a particular growth pathway without genetically changing the cells. Central to this process has been purifying and characterizing the active form of Wnt proteins, which had proven especially frustrating.

The use of conventional methods of isolation, such as that described in the prophetic example of Rodan *et al.* always failed to provide for isolation of the active protein. The only

reliable sources of active protein were the small amounts found in unfractionated culture supernatants or cells.

The reason for these failures remained an open question for many years. It was not until the work of the present invention that an answer was obtained. Unexpectedly, Wnt proteins were found to be much more hydrophobic than their sequence would suggest. This was due to modification of the protein by attachment of a lipid after it is produced. This means the protein was not soluble and would stick to containers, so standard purification techniques didn't work.

The inventors of the present invention developed techniques that used detergents to render the protein soluble, and also robust cell assays that would measure the biological activity of the protein. It was difficult and challenging to get the methods to work.

For example, one member of the Wnt protein family, mouse Wnt3a, is activated by the attachment of a lipid called palmitoyl to a particular amino acid on the protein. The lipid is necessary for activation, as is the presence of the attached amino acid, cysteine, which is conserved in all Wnt proteins. Therefore, methods of isolation that do not retain the attached lipid fail to provide for active protein.

Applicants respectfully submit that the cited art provides only a prophetic example with general guidelines for affinity chromatography. There are no guidelines in the cited reference that teach one of skill in the art how to perform affinity chromatography in such a way as to obtain active Wnt protein in a substantially homogeneous composition. Indeed, if the art had taught such a method, it would have been widely practiced by the many researchers seeking such compositions.

There are numerous unpublished tales of failed attempts to produce secreted Wnt proteins in cell culture. In general, overexpression of the genes in cultured cells results in accumulation of misfolded protein in the ER (Kitajewski *et al.* (1992) Mol Cell Biol. 12(2):784-90, copy attached). Secreted forms of Wnts have been found in the extracellular matrix or the cell surface (Burrus *et al.* (1995) Exp Cell Res. 220(2):363-73, abstract attached), but efforts to solubilize this material were not successful.

In short, the art worker has been motivated for a number of years to produce an active, substantially homogeneous preparation of Wnt, and has failed. Applicants respectfully submit that methods of isolated substantially homogeneous Wnt compositions that maintain activity of the protein are not taught by Rodan *et al.* Withdrawal of the rejection is requested.

Claims 7-11 have been rejected under 35 U.S.C. 103 as made obvious by the combination of Rodan *et al.*, U.S. 5,780,291 in further view of Lambeth *et al.*, U.S. 6,620,603, Vernet *et al.*, U.S. 6,653,448; and Matthews *et al.*, U.S. 6,159,462. Applicants respectfully submit that the presently claimed invention is not made obvious by the cited combination of references.

As discussed above, Rodan fails to provide a method whereby active and substantially homogeneous compositions of Wnt are obtained. The application vaguely indicates that affinity chromatography might be used, but fails to provide guidelines are demonstrations of an actual product.

The secondary references fail to remedy the deficiencies of the primary reference. While various methods of isolating proteins are known in the art, until Applicants' determination that Wnt comprises an unexpected lipid moiety, there was no motivation to isolate Wnt in a manner that would preserve its activity. Therefore, for many years researchers failed int heir attempts to isolate the protein, due to a lack of understanding of it's chemical nature.

As shown by the work of Matthews *et al.*, even with considerable resources and motivation, purified active Wnt was not to be obtained prior to Applicants discovery. As evidenced by the specification of U.S. Patent no. 6,159,462, at columns 23, line 39 to col. 24, line 10, the inventors believed that it was possible to isolate Wnt with a standard laundry list of purification methods, although no specific methods or results are provided with respect to this goal.

However, in the corresponding journal article published by these inventors, it can be seen that attempts to isolate active Wnt, even as a partially purified protein, were unsuccessful. As described by Austin *et al.* (1997) Blood 89(10):3624-3635 (attached), at page 3626, second column, under purification of gD.Wnt5a.His_6, the authors attempted to purify Wnt without including detergent to maintain the solubility of the protein. The article states that:

> *Purification of gD.Wnt5a.His_6* . Stable lines of CHOdp12 cells transfected with gD.Wnt5a.His_6pSVi.del.d were selected and maintained in glutamine-, hypoxanthine-, and thymidine-free media. Extracts were made from gD.Wnt5a.His_6 CHO cells as described above, and WNT-5a protein was affinity purified using 5B6-CPG essentially as described in Paborsky et al.[48] In brief, cell lysates were prepared as described above and were bound to the 5B6-CPG; the resin was washed extensively in PBS and eluted with acid. The eluate was neutralized, dialyzed against PBS, and refolded in 8 mol/L urea. The refolded WNT-5a protein was diluted in HSC media to a final concentration of less than 60 mmol/L urea in the flASK cell suspension culture assay.

This clearly indicates that the protein was eluted in phosphate buffer saline (PBS) in the absence of any detergent, and was refolded in urea in the absence of detergent.

The results of this attempt may be seen by comparing Figure 4, where conditioned medium from cells expressing Wnt was shown to be biologically active, and Figure 5. Following the conventional affinity purification scheme (shown in Figures 5A and 5B) there was a decrease in the contaminating protein present, and yet the biological activity, shown in Figure 5A, was not increased.

Applicants respectfully submit that the presently claimed invention is not taught or suggested by the combination of references. Prior to the present invention, one of skill in the art was uninformed as to the true molecule nature of Wnt proteins, and therefore could not select an appropriate method for purification of the active protein.

In view of the above amendments and remarks, withdrawal of the rejection is requested.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN-299.

Respectfully submitted,
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Date: July 7, 2005

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